NATIONAL ADVISORY COMMITTEE
ON MICROBIOLOGICAL CRITERIA FOR FOODS

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RISK ASSESSMENT ON THE PUBLIC HEALTH IMPACT

OF VIBRIO PARAHAEMOLYTICUS

IN RAW MOLLUSCAN SHELLFISH

Wednesday, May 26, 1999
8:00 a.m. to 4:10 p.m.

The Ambassador West Hotel
George I Conference Room
1300 North State Parkway
Chicago, Illinois

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I N D E X

P R O C E E D I N G S

DR. MORRIS POTTER: Okay. Good morning and welcome to the public meeting on Vibrio Parahaemolyticus Risk Assessment. I'm Morrie Potter from the Food and Drug Administration and I'll be chairing the public meeting. Mike Jahncke, the Chairman of the Risk Assessment Subcommittee for the National Advisory Committee will be chairing the actual risk assessment proceedings.

These meetings, which the FDA is holding in cooperation with USDA's FSIS, are about risk assessment to determine the prevalence and extent of consumer exposure to Listeria monocytogenes tomorrow and vibrio parahaemolyticus today.

The risk assessments will evaluate the resulting public health impact of such exposures.

The quantitative risk assessments of the prevalence and extent of exposure of these pathogens will provide us the structured approaches to synthesize and evaluate available data and information.

The goal of these risk assessments is to provide the regulatory agencies, FDA and USDA, with the information needed to review current programs relating to the regulation of these pathogens in foods and to insure that such programs provide maximum public health protection.

I'd like to turn the proceedings now over to Dr. Jahncke so that he can introduce the risk assessment itself. We will have two periods this afternoon for public comment. The rest of the time, when there are opportunities for exchange between the people presenting the risk assessment and the committee, if there is extra time, we will entertain comments from the floor at that point as well. Dr. Jahncke.

DR. MICHAEL JAHNCKE: Thank you, Morrie. I think what I would like to start with is just an introduction of the committee members around the table. My name is Mike Jahncke. I'm with Virginia Tech.

MR. DANE BERNARD: Dane Bernard with National Food Processors Association.

MS. ANGELA RUPLE: Angela Ruple, The National Marine Fisheries Service.

MR. MEL EKLUND: Mel Eklund, Mel Eklund Associates from Seattle.

DR. ROBERT BUCHANAN: Bob Buchanan, Food and Drug Administration.

MS. CATHERINE DONNELLY: Cathy Donnelly, University of Vermont.

DR. MICHAEL JAHNCKE: Okay. Remember that these proceedings are being transcribed, so please identify yourself when you speak and speak into the microphone. Thank you.

Let us formally begin the meeting then. All of you should have in front of you a draft agenda. The title of it is: "Risk Assessment On The Public Health Impact Of Vibrio Parahaemolyticus in Raw Molluscan Shellfish."

I believe we are on schedule. Dr. Potter has given the welcome introduction. Committee members along the table have introduced themselves. So, let's begin with our first presentation. Dr. Marianne Miliotis will be speaking on Introduction to Vibrio Parahaemolyticus Risk Assessment.

DR. MARIANNE MILIOTIS: Good morning everybody. I'd like to welcome you and thank you for attending our meeting on Risk Assessment On The Public Health Impact Of Vibrio Parahaemolyticus in Raw Molluscan Shellfish. I'd like to thank the organizers of NACMCF for giving us the opportunity to present what we have performed so far. I would especially like to thank Kathy De Rova (phonetic) and Linda Hayden (phonetic).

The meeting today is multi-factorial. We will explain to you why we're doing a risk assessment on vibrio parahaemolyticus and why in raw molluscan shellfish, particularly oysters. Dr. William Watkins will give you an overall background of the vibrio parahaemolyticus characteristics and current efforts and methodology.

He will also go over some questions that we hope to be able to address and the scope of the risk assessment.

After the break this morning we will get to the meat of the meeting. We will discuss the key parameters we have identified to be used in the risk assessment. I will then briefly summarize our approach and conclude with what we hope to achieve at the end of the day.

During the course we will also let you know what we, the risk assessment task force, expects or would like from you, the audience.

In the summer of 1997 the largest reported outbreak of vibrio parahaemolyticus in North America occurred in the Pacific Northwest associated with eating raw oysters. 209 cases were involved. This is ranging from California to British Columbia.

In 1998, there were more outbreaks in the Pacific Northwest, in the Gulf Coast, and in New York, all associated with consumption of raw molluscan shellfish, particularly oysters. This was the first reported outbreak of vibrio parahaemolyticus linked to the consumption of shellfish harvested in the New York waters.

This is just an example of one of the many oyster beds and harvest waters that were closed to prevent further outbreaks. In some cases some of the oyster beds were closed for at least six weeks.

In November of 1998, the Center For Food Safety and Applied Nutrition of FDA decided to conduct a risk assessment on vibrio parahaemolyticus.

In January of this year an internal task force composed of FDA employees was brought together to conduct this risk assessment. The charge to the task force was to conduct a risk assessment on the public health impact of vibrio parahaemolyticus infections caused by the consumption of raw molluscan shellfish.

The question has been asked several times, why risk assessment on vibrio parahaemolyticus and why in raw molluscan shellfish? Well, the outbreaks in 1997 and 1998, which involved over 700 cases, brought many factors and concerns to the forefront.

Firstly, the majority of the cases implicated molluscan shellfish, particularly oysters. Then we had these newly emerging outbreak strains. For example, 03:K6, formerly known to be associated with outbreaks in Japan and the Far East, and last year they arrived in the

United States both on the Gulf Coast and then later on in New York.

The current criteria that ISSC is using to close -- well, in the recent outbreaks, the criteria that ISSC used to close the harvest waters was based on illness. Reopening waters was based on two factors.

Firstly, changes in season or conditions, primarily temperature known not to be associated with outbreaks.

Secondly, absence of the outbreak strain.

Will this be effective in the future to prevent more outbreaks?

Another thing is, based on clinical studies conducted over twenty-five years ago, as well as investigations into outbreaks, this again, over twenty years ago, based primarily on cross contamination of cooked crabs, the FDA indicated that the levels of vibrio parahaemolyticus should not exceed 10,000 colony-forming units per gram. Is this adequate to prevent illness in the public?

The charge of the task force is to evaluate the increased risk due to newly emerging outbreak strains, current criteria for closing and reopening shellfish beds, the current FDA standard of 10,000 CFU/g and effectiveness of intervention standards.

This is our time line which we hope to achieve. As I said earlier, FDA decided to conduct a risk assessment on vibrio parahaemolyticus in November. The internal task force was brought together this year. In February we introduced the concept of our risk assessment to NACMCF. Today we are presenting the key parameters we identified to perform this risk assessment. We hope that in September of this year we will be able to bring to NACMCF the completed or draft version of our completed risk assessment. We will be welcoming comments and suggestions and our wish is to present the final risk assessment in November of this year.

What are we expecting from the working group today? Do we have a sound scientific approach? What other data do we need? Do you have information for us? What are we expecting from the public? Any comments or suggestions, and as we requested both in our Federal Register Notice, and I think you may have the document of the risk assessment of the parameters we have identified, by July 6 we would welcome any pertinent information you may have that you think would help this risk assessment along.

Any questions?

DR. MICHAEL JAHNCKE: Thank you very much.

DR. MARIANNE MILIOTIS: I would like now to introduce Dr. William Watkins.

DR. MICHAEL JAHNCKE: Dr. Watkins will be giving some background on these issues.

DR. WILLIAM WATKINS: Good morning, everyone. On our schedule I see I'm scheduled to give you all this background information in fifteen minutes. But, we're well ahead of schedule so it will take a little longer.

I want to cover just a little bit about vibrio parahaemolyticus. We are all here because we did have the recent outbreaks and this is a new phenomenon for this country, outbreaks transmitted by the consumption of raw oysters. We had indications this may have happened in the past, but never to this extent, and not really very well confirmed, at least not the regulators.

So we have an organism that we need to understand right from the beginning is naturally occurring. This is not an organism that is something we've encountered in the past transmitted by fecal contamination. It's not like salmonella, which grew our National Shellfish Sanitation Program.

We had over 700 cases, as Marianne has said, in the last two years. This is not a small number for shellfish. We're going to hear more about these outbreaks and other outbreaks later on.

I just want to mention briefly for you some of the disease syndromes that are caused by this organism, its characteristics, a little bit about its pathogenicity and ecology, its epidemiology, which you will hear quite a bit of later on, and something about the current scientific efforts that are going on to address the questions that we cannot answer at this time, particularly some work going on for methods development.

As I mentioned, it's not associated with a fecal source, so we have no way to index it. The fecal coliform or total coliform groups which we use to index fecal contamination which carry the other bacterial pathogens don't help us in this situation.

Vibrio parahaemolyticus has been known for quite a long time. It was discovered in 1950 in Japan with an outbreak caused by the small, half-dried sardines, where 272 people became ill with gastroenteritis and 20 of these individuals died. So, it was known right from the onset this could be a fatal outcome.

Remarkably, vibrio parahaemolyticus continues to cause somewhere between 40 to 60 percent of the illnesses, the gastrointestinal illnesses that occur in Japan today.

It hasn't gone away, and we can look at some information from Japan to get a little bit of an idea of how they experienced this.

It's not surprising though, because Japan's annual consumption of seafood is quite a bit higher than our own.

The organism causes two disease syndromes. One of these is septicemia. That's simply an infection in the blood. It causes extensive tissue damage and death can result. Often when this syndrome occurs the organism enters an individual's blood by wounds that an individual either has or experiences at the shoreline, in the water, or working with seafood.

However, the organism can also enter the blood after being ingested, so it is not unlike vibrio vulnificus. In certain high-risk consumers we find this can be a very bad outcome.

This is just a complete report from a Dr. Roland. The first one I remember was a leg gangrene case of a man clamming in Narragansett Bay. He experienced a wound. It got into the blood. He lost his leg because of this. A lot tissue damage goes on with these organisms because they have a lot of enzymes that can destroy tissue.

What is vibrio parahaemolyticus? Obviously, it can cause acute gastroenteritis. We've had many cases of that. We'll get a lot more information on the outbreaks later on.

It is a gram-negative organism, that simply means it stains gram negatively in the classic gram stain. It's rod-shaped. It is found in the estuarine environment, not very commonly isolated in the fresh water or the open ocean environments, simply because it requires salt to grow, so it's not going to be found in fresh water unless it's associated with organisms. And, it's not normally found in the open ocean. I'm not sure really why. It does tolerate those salinities. But, out there there's thirty-five parts per thousand. It seems to be simply an estuarine organism. This is where it thrives.

It is ubiquitous. We can find this in all of the coastal and estuarine waters around the world. I don't know of any that it hasn't been found where it has been looked for. It is halophilic. As I mentioned, it requires salt to grow. This is an important factor to remember because for many years our clinical laboratories did not have media that was supplemented with salt to grow it. So, often times when cases came it's quite likely that we did not record those as vibrio parahaemolyticus cases. Because the laboratory did not isolate the organism, it wouldn't grow.

Because it requires salt it can be distinguished from organisms that are very similar in biochemical characteristics like aeromonas, which do not require salt.

It's very metabolically diverse. It uses a wide variety of carbohydrates for carbon and energy. One of these starch hydrolysis has been used in the laboratory to separate it from other bacteria. It's one of the distinguishing characteristics.

Vibrio parahaemolyticus is what we call a facultative anaerobe. It grows aerobically, so it can use oxygen as the final electron acceptor and it tests as cytochrome oxidase positive, a simple lab test that also helps to distinguish it. But, it preferentially ferments carbohydrates. That is, it will produce acid and lower the pH of laboratory media when it uses carbohydrates. This we can use to help distinguish it in its fermentation patterns.

But, what makes it a little bit different than a lot of organisms is that it does not produce gas when it ferments carbohydrates. It's anaerogenic. So, unlike the classic MPN for coliforms where you look for gas production from lactose, this organism, number one, doesn't use lactose, I don't think, and number two, it does not produce any gas. So this helps us to differentiate it from the anaerogenic fermenters, which are very numerous.

Vibrio parahaemolyticus grows in liquid media and has a single polar flagellum. That's kind of unusual too, so that's another characteristic we have used in the past to make certain that we were dealing with this species, particularly before the advent of DNA techniques.

The organism prefers alkaline pH's. It can tolerate pH's as low as about 4.8 and as high as 11. But, its optimum range is about 7.5 to 8.6 pH. This correlates pretty well with the pH of sea water, which is around 8.5 in most cases. Estuarine waters are comprised in large part of sea water.

I mentioned that vibrio parahaemolyticus is a halophilic organism, requires salt for growth, and some of the early work tested all kinds of salts. The optimum range for the organism's growth is between 2 and 3 percent. Here you can see its salt in terms of molarity and about a half a molal, a .5 molal is about 2.9 percent salt in the medium, sodium chloride. We've used this characteristic to help distinguish it from other organisms that are closely related as well. For example, vibrio alginolyticus in the environment outnumbers parahaemolyticus by a large amount. Most of the time when parahaemolyticus is present we can distinguish isolates of alginolyticus, which can grow at 10 percent salt. Vibrio parahaemolyticus can grow up to about nine, but not at ten percent.

So, it can't grow at zero and it can't grow at ten, and that's pretty much its range for growth, low percent up to ten percent. Optimum between two and three. This is important to know, because you need the salt and the medium to give it an advantage to grow so that you will detect the organism.

With regard to temperature, vibrio parahaemolyticus is optimal growth between 35 and 37 degrees. It has a range of about ten degrees Centigrade to 43 degrees Centigrade. I've seen reports that indicate some strains can grow lower, some strains can grow higher. But, at 35 or 37 we're talking about body temperature. So when it comes from the environment, even if it's warm out at 25 or 30 degrees Centigrade, and goes into a nice warm body, which is optimum for its growth, it's easy to understand why this organism can grow rapidly and cause us a problem.

It is a rapid grower. It is one of the fastest growing organisms that we have on record. The generation time for this in ideal conditions in the laboratory has been reported to be ten minutes. I've even seen one report indicating 8.5 minutes. That's very close to the theoretical maximum for bacteria. So, you can see that this organism may have a distinct advantage over some of its competitors in the environment when conditions permit, because it can grow so rapidly. It uses carbohydrates very rapidly in its fermentative growth, and it competes very well.

Just a little bit about its ecology. As I mentioned, it is naturally occurring, and I mentioned its temperatures of preference. It has, apparently, a seasonal cycle. It grows and thrives in the warmer months of the year. It doesn't lend itself to easy detection when the temperatures fall below 14 degrees Centigrade in the water. You can't find it in the water column very easily at all.

You can detect it sometimes in the sediment. You can detect it sometimes associated with fauna, but whether it's present in over wintering sediments or in fauna or goes into a viable non-culturable state, it's not really clear, simply that in the warm weather that's when we are presented with the problem.

Vibrio parahaemolyticus has an enzyme called chitinase. That means it can hydrolyze chitin. Often times we find it associated with zooplanktons, which have exo-skeletons of chitin. During the warmer months they probably colonize the zooplanktons and that may be a means of how they get into the shellfish. Shellfish are filter feeders and strain out algae for food and at the same time they take out some of the zooplanktons.

It has been found associated with the intestinal tract of fish, so it's easy to understand how this organism is dispersed throughout the coastal waters and it spreads around the world. Again, it is not sewage-related, so its occurrence is not indexed by indicators.

The pathogenicity of the organism is derived, at least in part, from some of the enzymes the organism produces. These are biologically active compounds with some toxic effects. There are several compounds that have been identified as cytotoxins and several hemolysins. We are able to make use of some of these hemolysins in our ability to try and distinguish these species from others in our identity.

One of the hemolysins produced by all strains of vibrio parahaemolyticus is a thermolabile hemolysin. That is an hemolysin that is sensitive to heat and abbreviated the TLH hemolysin or the thermolabile hemolysin gene. We can use that then as a good species-specific marker with our gene-probe techniques.

There is another distinctive feature of this species, however, and that is, it's not unique, but it's distinctive, not all strains are pathogenic. In fact, when we go to the environment and try and isolate pathogenic strains we have a very hard time finding them, for the most part. It appears that the vast majority of strains do not cause acute gastroenteritis, are not, in fact, truly virulent.

So, how can we distinguish the virulent strains from the normally benign strains?

There is another hemolysin, a thermostable direct hemolysin, abbreviated TDH in our designation of the gene, and the TDH hemolysin, TDH gene, is a characteristic that correlates very highly with the clinical isolates, those that we have isolated from patients. About 97 to 98 percent of the patients' strains are positive for this characteristic, a hemolysin that enables blood cells to be lysed, that is fresh uncitrated blood cells in a very high salt, high alkaline medium called Wagatsuma agar. This was called the Kanagawa phenomenon. The Kanagawa phenomenon correlates very highly with our clinical isolates. With the environmental isolates very, very rarely is a Kanagawa positive strain isolated. I think you'll hear a little bit more about this phenomenon later on, and I think you'll see it in slides as KP positive or negative.

I just wanted to mention that vibrio parahaemolyticus in addition to being toxic, toxigenic, can also be rather invasive. There are many times we've seen micrographs of the intestinal walls of oysters where it's behind the epithelial layering in humans. It can penetrate the lamina propria layer, and so it's not surprising then that the gastroenteritis caused by vibrio parahaemolyticus is very often accompanied by some severe epigastric pain, not a mild disease.

We use serology to differentiate these strains as well. We've got Kanagawa phenomenon, and all these biochemical characteristics, and we now have gene probes. But, to look at strains, like with other pathogens, we often look at the somatic or cell wall type antigens and produce antisera against these so we can characterize them by their O antigens. We also have capsules that differ among various strains of vibrio parahaemolyticus. So we can use the capsule or the K antigens.

So, as you saw before from Marianne's slide, you saw an O3:K6, that is a serotype that was associated with two of the outbreaks actually, and we use that to try and trace, figure out, what's going on when patients become ill and how many different strains are involved in an outbreak. And, perhaps some day where they come from, why they are there.

The organism also has a flagella, as I mentioned. Flagellum and flagella, when it's in other media it can produce multiple lateral flagella, as the slide showed from Marianne.

These are antigenic as well, but the H antigens, as they're called, don't help us to distinguish species, so we pretty much don't use them for that purpose.

I just was going to mention real quickly some of the epidemiology that we know. The organism has caused outbreaks that have been seafood and shellfish related all around the world. This was some information from Taiwan over a ten-year period, and the VP is for vibrio parahaemolyticus. The other two, Staph aureus and Bacillus cereus were the other organisms that were most prevalent associated with the gastroenteritis in Taiwan.

If we look at the next one it shows the occurrence of the outbreaks by month. We get kind of a hint that the warm weather months are a big problem for parahaemolyticus, not so much the colder months. But, that's sort of also true for the other agents of gastroenteritis as well.

In Japan, on the next one, you can see that -- I think this is 1994/1995 information, where they plotted the cases of vibrio parahaemolyticus illness. Now, mind you, that's not necessarily just from raw oysters, that's from all seafood. But, it does have a seasonal kind of occurrence. We see the two very distinct peaks here for those years.

So we know all this. Why are we surprised by the last two years' outbreaks? Well, in the next one, this was our record. I hope you can read a little bit of that. This was a table summarized by Barker in 1974. It relates to the cases that we had in the late sixties and early seventies. We did have some outbreaks, but they were, for the most part, not borne by shellfish. Steamed crab was associated with this organism a lot, and that was presumably due to re-contamination, and also to not storing the food after it had been re-contaminated, so that vibrio parahaemolyticus was now allowed to grow and caused quite a bit of illness. A large number of cases. So, this got our attention.

Down below there were some unconfirmed

outbreaks, as you can see. One with roasted oysters from Washington, and another in Texas in 1971, associated with raw oysters, but they were not confirmed. Parahaemolyticus wasn't isolated from the patients. It's just that the symptomology seemed to be consistent with a parahaemolyticus illness.

Very often when we go back to the foods we can't incriminate them very easily. We have a difficult time time isolating the Kanagawa positive strains from those.

We had one other indication in the next slide. This was reported by Kaysner and others in 1981. A very small raw oyster outbreak, six individuals in Oregon and Washington. So we had kind of an idea maybe this could be a problem, but in the absence of repeated incidents there was no major attention drawn to this organism as a big pathogen. We had simply a whole case record of sporadic illnesses.

In the next slide, the 1970's and 1980's that's a bad scan, but that's kind of what our record of parahaemolyticus looked like in the epidemiological side of things, just sporadic cases caused by various seafoods here and there. This is pretty much what our opinion of the organism was. It was occasional. It wasn't very frequent, and the outcomes usually weren't too severe.

So, as you can see, vulnificus was involved more often than parahaemolyticus.

Again, in the next slide you can see that it didn't just happen in our warmer climates. It happens in our Mid-Atlantic States, and actually just about everywhere, Washington up in the northeast. If you look real hard at the epidemiological record, and some people did, they summarized things like this. In the Chesapeake area for about a twelve-year period or so, fourteen years, parahaemolyticus cases, they can find them, they can document them, along with other vibrios. It's more prevalent than the others.

In the next, we can see what that kind of case record looks like. Again, sporadic cases occurring once in a while, not necessarily even every year. So, no real evidence of this organism as a big threat to cause outbreaks.

In the next one we can just get an idea from Florida data, again over that previous seventies and eighties, how the vibrio illnesses seem to sort out mostly in the warm weather months, and even in the south and the Gulf Coast.

In the next one, again just a couple years of the data showing that the warmer months are much more of a

-- well, it's not so much obvious here. Parahaemolyticus though in these two years does cause more illness than any of the others that they documented. So, it's pretty prevalent. I don't know if things are changing or not.

In the next one we see the kinds of information we attempted to gather early on. This is a volunteer study. I say volunteer kind of with tongue-in-cheek. These were graduate students of one of the professors in Japan, where they were fed suspensions of the organism. If we just extrapolate to what densities of bacteria can be in a test tube when we grow them up they can be anywhere from about five times ten-to-the-ninth to a little over ten-to-the-tenth. So a one to ten thousand dilution of that sort of suspension leaves us with somebody swallowing up to several million of these organisms. Maybe as few as a half-a-million. So that's the kind of information that was gathered to try and find out what levels of this organism caused the illness.

From the 1997/1998 outbreaks, which we'll get a lot more information on later, several shortcomings were pointed out to us, at least from the 1997 outbreak. One of these had to do with our methodology. We had been using an MPN procedure, which is very standard for most foods, that involved an enrichment and a streaking and sub-culturing and biochemical identification of the isolates. It was kind of cumbersome. It wasn't very rapid and it wasn't always that reliable. There were some problems with it itself.

We also could tell from what was going on with the 1997 outbreak that a lot of times the reports of illnesses didn't reach the public authorities until three weeks later or more. So our reporting system wasn't exactly responding in a real timely manner. This is not uncharacteristic sometimes in food outbreaks. But, it sure would help if we could get the information more quickly if we're going to address this in any other way than we have been.

The third shortcoming, if you will, was how the shellfish program dealt with defining an outbreak. It was unclear how to designate what area would be implicated. With an outbreak that is caused by hepatitis or salmonella, any other bacterial-acute illness that comes from a fecal waste we can try and identify that fecal source and correct the matter. In the meantime we can close the growing area, the shellfish area that's designated by its sanitary characteristics for harvest until the problem has been corrected.

But, with a naturally-occurring vibrio

parahaemolyticus do we close the lease site that was implicated or do we have to close the adjoining lease sites, or is it the growing area for all of the lease sites, or is it the adjoining growing area, or is it the entire bay, or is it the entire region? Difficult questions. We don't have the answers to those things. But, our definition of an outbreak, specifying a closed area, needs to be examined a little bit.

Another shortcoming that maybe is indicated is a lot of times people's histories involve eating not just oysters, but also clams and other seafoods, and how do we sort that out? Is it just oysters and clams when we have an outbreak? Some of the outbreaks are very clearly defined by oysters being eaten raw. But, clams are also harvested in some of these areas in the Northeast for example. Do we have to prevent the harvest of all animals, even though the clams may not be implicated?

Another factor that seemed to give us pause was the fact that sometimes an outbreak can be caused by what appears to be a single strain. That is an 03:K6 serotype. So, do we define then an outbreak caused by a single strain, as it was in the Gulf Coast and Northeast outbreaks in 1998, or do we keep it to all or any pathogenic or virulent strains that are found as it often happened in the Pacific Northwest in 1997 and in 1998?

What time factors should we consider? Does an outbreak have to be defined by illnesses that have been occurring just a few days apart or a week apart, or a month apart? Or, maybe a whole season, because this is a naturally-occurring organism. Its presence and absences is not something we can control, nature is doing that, the organism is doing that. So how do we define what time factor is most appropriate?

Lastly, we had some guidance, as Marianne had mentioned, we had 10,000 vibrio parahaemolyticus per gram as guidance for not allowing that to be served. How adequate or inadequate is our guidance and how could we improve that? We had some shortcomings, we think, there. We had some indications that far fewer of the organism may be needed to cause illness.

Well, there are some current activities, if we could look at the -- one of these has to do with methodology. Recognizing that our standard MPN procedure represented under BAM, which stands for the Bacteriological Analytical Manual that is put out and published by FDA, it's a compendium of methods that we recommend to our field laboratories to use when they're trying to deal with detecting pathogens usually, sometimes quantifying them. This is very characteristic of most foods. There are MPN procedures for pathogens. We use an enrichment step to get everything that may be there to grow, giving maybe perhaps a little bit selectivity. So in this case we use alkaline peptone water incubated at 35 degrees Centigrade. Following that we streak suspect colonies onto a TCBS -- I'm sorry, streak some of the growth in the tubes onto TCBS plates, which stands for thiosulfate, citrate, bisalts and sucroses as parts of the ingredients of that medium. It's a fairly selective medium. It has oxgall as a bisalt and it's pretty harsh. It doesn't allow very many of the background to grow. That's incubated at 35 degrees and typical vibrio parahaemolyticus colonies are sucrose negative so they come up green. Whereas, those that ferment sucrose turn the pH to a lower acidity and they turn orange or yellowish in color. That's vibrio alginolyticus, for example. So, you pick suspect colonies and then you must purify them and test them biochemically. That's what we've been doing. It takes quite a while. It's got some disadvantages in terms of time, cost, pretty labor-intensive, and not always successful, because the alkaline peptone water may produce a lot of other organisms that grow and out-compete the parahaemolyticus, and you don't get them on the streak plates.

Today we're trying to develop, it's still being worked on, but it's been fairly successful up until now, a simple straight spread-plate method using gene probes to identify both the species and the virulent strains of the vibrio parahaemolyticus. So, we have a very non-selective plate media, just tryptone, salt and auger, T1N3 plates. They're spread plates. From these, after growth, you can lift some of the growth from the colonies on different membrane filters, filter papers. You treat these to open the cells up, clean up some of the protein and then allow DNA hybridization to occur with a probe that is specific for the genes of choice. In this case we are using the thermolabile hemolysin for the species count on one of the membrane filter lifts and a probe for the thermostable direct hemolysin, the TDH gene to determine the number of virulent strains or pathogenic strains that are there. This relates to the Kanagawa phenomenon.

Let me go on here. It's far more rapid. Its recovery is fairly good, as it is with the MPN, but it makes a great big improvement on precision. With MPN's or most probable numbered procedures, you have to realize these are statistical estimates of the bacterial population that you're looking for. As such, because they are statistical estimates, they have historically, or characteristic of them, there is very poor precision. That is, they have a very, very wide confidence limit. The 95 percent confidence limits of the 3 and 5 tube MPN's is so broad that you don't often want to hang your hat on the number that you're generating.

With a direct plating procedure, or with most direct enumeration procedures you can improve the precision of the method to about plus or minus 20 percent, plus or minus 30 percent, sometimes tighter than that. So you have a lot more confidence in the number that you are reporting.

One advantage of the MPN procedures is that they have an ability to have sensitivity. We can measure a large amount of the substance we're looking at, the food. With the direct plating procedure right now we're limited in that sensitivity. We generally look at one-tenth of a gram. That allows us to detect vibrio parahaemolyticus when they're present at a level of ten per gram or greater. Not too sensitive. We can make some improvements on that and I think that sooner or later we will.

In general, the direct plating procedure we're developing is faster, it's cheaper, and I think it's probably more reliable. It certainly is more precise.

Let me move on. Just to show you what the direct plating procedures consists of with the probe, you blend the sample in a buffer, a phosphate buffered saline. Make dilutions. Spread those dilutions on the plates. Incubate the plates. Allow the organisms to grow. Make colony lifts on filter paper. Lyse the cells to expose the DNA. Clean it up with some wash. Hybridize with the DNA probes that are specific for those genes, and then allow the signal from the alkaline phosphatase or the Dig probes to develop, and you get a signal. You get a little color change and you can make an easy colony count right off of those membranes.

The next one is just a little bit more detail on how we go about that.

Some other things that are going on, just to mention for current activities. We have been engaged in a study of the retail oyster and clams at the marketplace. I think it's principally oysters at this time that we're looking at. That is due to finish up in about a month or so. I think you will hear some data from Andy DePaola on this later. But that will essentially, hopefully, tell us a little bit about what the vibrio vulnificus and vibrio parahaemolyticus levels are in oysters at the marketplace just before people are about to consume them. So what is their exposure, in other words.

We are also embarking on an oyster harvest study. One which will look at the levels of parahaemolyticus in oysters out in the growing areas before they are harvested. So we will have numbers, levels, if you will, from both ends of this, right before consumption, and before they are even harvested.

Right now we are embarking on this Food Safety Initiative Risk Assessment to try and assess what we will need to know to deal with this problem in the future.

There have also been some other intervening measures that are being investigated by various people. In the past you probably have heard about gamma irradiation of shellfish to see if they can reduce the bacterial loads on oysters and clams. That works pretty well for bacteria. It doesn't do much for viruses. There are studies that have been reported on and people investigating the use of high pressure treatment of shellfish to reduce bacterial levels. There are people who have worked and have reported are now using pasteurization and freezing to reduce bacteria levels in shellfish. All of these intervening measures --

DR. MICHAEL JAHNCKE: (interrupting) Dr.

Watkins, you have to wind up your time.

DR. WILLIAM WATKINS: Okay. All of the intervening measures may -- some of them may prove to help us out here. Depuration has been investigated, it hasn't worked too well in the past. So we have quite a bit perhaps yet to learn. Hopefully this risk assessment will tell us that.

That will wind up the background presentation. Any questions or comments on that?

MR. MEL EKLUND: Yes. I'd like to ask a question. Mel Eklund. During many of the outbreaks, as you had mentioned, a lot of times when they go back to look at the levels of vibrio they found it to be very low. I have often wondered, with your MPN procedure what role the bacteria phages may play in this. A list back in 1978 showed that there was a lot of virulent phages present in these environments. During an enrichment procedure like this I often wondered how many cells are actually lysed by these lytic phages during the enrichment procedure. Would you comment on that, please?

DR. WILLIAM WATKINS: Well, I certainly have no idea how that may or may not have effected the numbers that the procedure actually winds up determining. If that were the case, I'm not sure why, we would find that true in foods that are incriminated in outbreaks, but not in all instances. If the phage are that prevalent in environments where vibrio parahaemolyticus resides, I would think that we would see that over and over again. That may be the reason we don't find the virulent strains. They may be much more susceptible to phage attack and they are simply lysed and we don't streak them out.

I think perhaps though also we have to realize that the streak plate was never a technique intended to verify or confirm the presence of a bacterial isolate from a test tube in a mixed population. It was a technique designed to purify cultures so that we could pick a single colony, presumably derived from a single-cell that was planted in that site, and then doubled, quadrupled, and so on.

If you have a mixed population in a test tube and your parahaemolyticus virulent strains are outnumbered a thousand to one by the non-virulent strains, then you probably will never ever see an isolated colony, let alone be able to pick one off a streak plate. Because the best streak plates I've ever seen are about 200 isolated colonies. So you could pick them all, and we only really typically pick two or three. The virulent strains would be present, but they would be buried in the mass of growth around the parameter of it.

That might be more of what we're seeing going on. It's not -- I don't know the answer to that though, really.

DR. MICHAEL JAHNCKE: Any other questions? Before we do, I do want to remind the committee that we are here for risk assessment and we need to keep our questions focused on that. Bob?

DR. ROBERT BUCHANAN: I'm not sure you're going to cover the questions I have later on. I realize you are providing an introduction. So if you are, just so indicate.

Of the cases that you've seen, approximately of the oral cases, I'm not really interested in the wound cases, of the cases you see of orally transmitted vibrio parahaemolyticus, approximately what percentage are septicemic?

DR. WILLIAM WATKINS: I think Marianne Ross is going to give us quite a bit of detail on that later on, is that true? Yes.

DR. ROBERT BUCHANAN: Okay. Again, she may cover this later on. Of the fatalities, do you have any idea of approximately what percentage of these people have some underlying condition?

DR. WILLIAM WATKINS: I think she'll provide what we have on that too.

DR. ROBERT BUCHANAN: Okay. A little bit about the physiology of the oyster, the organism you're assessing. It is unusual among seafood in that it has a fairly substantial glycogen store, and typically upon holding the organism for any length of storage, the pH in the oyster decreases down to below the range where I believe that vibrio would actually grow. Is there any indication at all that the oysters that were associated with any of these outbreaks were for some reason glycogen depleted?

DR. WILLIAM WATKINS: I am not aware of anybody who has reported on that.

DR. ROBERT BUCHANAN: Do you have any data on what was the pH of those oysters?

DR. ANDY DEPAOLA: Yeah, Bob. Andy DePaola. We analyzed a lot of the samples on Dolphin Island and while we didn't test the pH, we have been conducting storage studies, and as store to sell stock we usually don't see pH's below six. They get to the low sixes, and the vibrio parahaemolyticus seems to do quite well at those pH's. That's after storage for over two weeks.

DR. ROBERT BUCHANAN: Is that unusual or is that restricted to Gulf Coast oysters? Because I know oysters taken from waters in the northern part of the country, and again, I'm not an expert on molluscan physiology, but my own measures I've taken indicate that it drops substantially lower than that.

DR. CHARLES KAYSNER: Chuck Kaysner, Food and Drug Administration. On the Pacific oyster we have seen the same thing that Andy has mentioned. As long as the oyster is alive the pH stays roughly around to the low sixes after storage. It's once we shuck the oyster and the animal dies that we start seeing the glycolysis and the lactic acid production, and then the pH drops quite dramatically, overnight we can get down into the range of four, which kills off the vibrio and a lot of the other bacteria.

DR. WILLIAM WATKINS: I think there may be a very few cases of illness transmitted by shucked oysters, but they are far and few between. The raw oysters have been the problem.

DR. ROBERT BUCHANAN: So all of your outbreaks were associated with live oysters.

DR. WILLIAM WATKINS: Oh yes, in the last two years in this country, absolutely.

DR. MICHAEL JAHNCKE: Any other questions? If not, Dr. Watkins has a second presentation, questions to be considered and scope.

DR. WILLIAM WATKINS: So, just to go over these real quickly. We have some questions that our risk assessment is asking and we hope to have either answers for these or ways to get at answers for these.

We ask, what are the frequencies of the virulent strains of vibrio parahaemolyticus in shellfish waters, and for that matter all vibrio parahaemolyticus if the total population would turn out to be a good indicator for us and easier to measure?

What are the frequencies of these in shellfish meats?

We'd like to know what parameters can predict the presence of virulent strains in waters and/or meats so that we can perhaps get a handle on this without having to go look directly for those virulent strains, which up until now it has been fairly difficult for us to detect, and if we do detect them, we're not, at this time, certain how to handle that information, unless that strain is identified in an outbreak. So how can we predict the presence of these strains?

We also would like to know how the levels of vibrio parahaemolyticus in the shellfish at harvest compare with the levels at the time of consumption. Is there something that's going on between when we harvest and when they reach the consumer, and does that matter, or doesn't it?

Obviously, it would be important for anybody to know what is known about this dose-response. What is known about the numbers of parahaemolyticus, and the strains of parahaemolyticus that can cause illness in people?

You saw earlier that there have been that and several other attempts to get at the so-called infectious dose or the level that causes illness, a number of volunteer studies. But, as you might guess, it's kind of sketchy as far as the information goes, and it doesn't give us, perhaps, quite enough. Maybe there's enough out there, I'm not sure.

How does the dose-response vary for the different strains of vibrio parahaemolyticus? Are all strains that are virulent created equal, or is one, such as 03:K6 or 04:K8, both of which have been found in certain outbreaks repeatedly, not just in this country but in other countries, are they more of an epidemic-type strain? Are they much more virulent? Are they going to require far fewer, or if not far fewer, do they cause much more severe of an outcome?

How does the dose-response vary among the humans with different susceptibilities? We see this plays a very large role with vibrio vulnificus. Is there such a factor related with the parahaemolyticus gastroenteritis and/or the septicemia that may result from ingestion?

What are the impacts of our post-harvest handling of shellfish? Simply put, the industry employs a wide variety of post-harvest practices. They vary a great deal in what happens to the shellfish. It would be important to know if this is a factor in outbreaks that occur, and if it is, what can we do to change that outcome.

What intervention-type strategies can be used to reduce the levels in shellfish? I mentioned just a few real briefly, gamma irradiation, pressure treatment, freeze/thaw, and pasteurization. People have tried depuration. Are there any other intervening strategies that might be useful to reduce the levels of natural-occurring vibrio parahaemolyticus and other vibrios, for that matter?

Is the current scientific knowledge adequate enough for us to reliably assess this risk? It may just turn out that what we'll find in the risk assessment that there are some important pieces of information that we need before we can do a reliable risk assessment, and we will need to go out and gather more information.

That being the case, where should that future research be directed? What are the questions we need to answer and how can we go about getting those answers?

So those are the questions, the kinds of questions that this risk assessment is asking. Does anybody have any comments on those, or questions? If not, I'll move on to the statements of scope.

The scope of the risk assessment is, I think, fairly simple, and yet complex. We would like to determine the relationship between molluscan shellfish, vibrio parahaemolyticus, and illness. Obviously, we know parahaemolyticus and other vibrios reside in molluscan shellfish as probably part of their normal flora, and that certain seasons are involved. But, I think we need more definitive information on the relationship, that will enable us to prevent illness.

We would like the risk assessment to assess what the human exposure to vibrio parahaemolyticus via the consumption of raw shellfish is.

I mentioned that we are doing a retail study at the moment. Those kinds of information may help there.

Next, we'd like to produce estimates of illness for levels of vibrio parahaemolyticus consumed by different sub-populations. If we have an exposure what can we expect the outcome to be?

Lastly, the scope, hopefully, will provide the kind of information that can be used for decision-making in either assisting industry or industries yet to be created, or regulators doing everything they can to prevent outbreaks of illness.

That's our questions and our scope of the assessment. Questions or comments from anybody?

DR. MICHAEL JAHNCKE: One question, is the scope of what Dr. Watkins presented clear to the committee; what we will be doing today, this morning and this afternoon?

I have one question for you. What is the ability of -- I know that various federal labs and others have the ability to identify virulent strains. What's the ability of some of the local states?

DR. WILLIAM WATKINS: It's growing. But, it's limited, I think. Practically anyone can do a Kanagawa test if they get a source of fresh uncitrated blood. It's a very simple test to do. So you can determine whether you have a virulent or Kanagawa positive strain very easy. There are only a few places that can do the serotyping, and I think there are other ways that we're going about trying to differentiate the strains that come out of this. One is -- oh boy. Well, there's certain gel electrophoresis patterns that we're looking at. A couple of states are doing that. But for the most part, I think the state and clinical labs that encounter the specimens from patients are pretty limited in what they're able to do.

DR. MICHAEL JAHNCKE: Andy?

DR. ANDY DEPAOLA: Yeah, Mike, Andy DePaola. The status of the state's capabilities to determine levels of pathogenic organisms has been growing, as Bill mentioned. In December, New York, Connecticut, and Texas were trained on DNA probe methodologies. Then about two weeks ago we had representatives from New Jersey, Maryland, Virginia, and Washington. In two weeks we will work with people from Louisiana, Maryland, and then the last week in June Chuck will be having a workshop out for west coast and Canadian shellfish people. This is all with non-radioactive DNA probe methods for best speciation and thermostable direct hemolysin.

DR. WILLIAM WATKINS: So the short of it is we're trying to do the technology transfer as we go, even really before the method may be in its final phase, final state.

DR. MICHAEL JAHNCKE: Bob?

DR. ROBERT BUCHANAN: Yeah. You've mentioned both the increased activity in training the states, and you've also, at least briefly, introduced some future plans you have. In those future plans are any of these likely to generate data that would be pertinent to the current risk assessment that you're conducting?

Can we anticipate any of those being completed to materially change the data you have on hand?

DR. WILLIAM WATKINS: I believe that may be a possibility. It's our hope that we will gather information from the oysters in their harvest areas in the environment.

DR. ROBERT BUCHANAN: And those surveys are to be completed before July 6?

DR. WILLIAM WATKINS: Well, no. They will be ongoing throughout the year, as I understand it. But, the early part of the summer will be done probably by mid August. No, I'm not sure that it will be helpful to the risk assessment effort here. Maybe some of the data will be available by then. Andy, do you know?

DR. ANDY DEPAOLA: Yeah, these studies are directed specifically at our gaps of knowledge for risk assessment. In the past there's been a lack of systematic, well-designed studies with effective methodologies to determine either total or the incidents of pathogenic strains.

In Alabama we began in March. I think the other states will begin early December and we plan to go at least one year.

DR. ROBERT BUCHANAN: Again I'll ask you the same question. Will any of these data be available and be pertinent to the current risk assessment? Will they be available before July 6? That's the date that it was earlier indicated that you will be closing the data gathering for this risk assessment. Will any of these be available before then?

DR. WILLIAM WATKINS: I do not believe so.

DR. ANDY DEPAOLA: We have data from Alabama that started in March and up until July that will be available.

DR. ROBERT BUCHANAN: Okay.

DR. MICHAEL JAHNCKE: Yes?

MR. KEN MOORE: Ken Moore, Interstate Shellfish Sanitation Conference. To help capture some of what you've heard, I don't want anyone here to get the impression that this is strictly an FDA effort. The ISSC has provided funding for much of this activity. The states are playing an integral role in it, in collecting this data. There's already been a great deal of data that has been collected, and to answer your question specifically, we're in the process of putting a technical work group together of the states that have been involved in the outbreaks. We're hoping to have maybe a case study report of the states that were involved last year with Washington, New York, and Texas. We're hoping to have a report available. It could be made available by July 6, I think.

But, this is a much bigger effort, quite frankly, than FDA. I mean, parahaemolyticus is being recognized by everyone involved in shellfish as a significant problem, and it's being treated that way by the ISSC, the states, and FDA.

DR. MORRIS POTTER: I appreciate those comments, Ken. There will be an opportunity after lunch for public comment. I'd like to remind you the ground rules of the discussion this morning is that it's an opportunity for members of the subcommittee first. NACMCF second to interact with the speaker, and we'll open it up a little bit more this afternoon. Since we are running a little bit ahead, however, let's go ahead with Marianne Miliotis for the first presentation and then we'll take a break.

DR. MARIANNE MILIOTIS: You will have to excuse me. Those of you who have children, especially young children, will know how they love to share things with you. Well, my voice today is the result of my four-year old's willingness to share everything with me.

The factors that determine whether vibrio parahaemolyticus in raw molluscan shellfish is a hazard include the level of the pathogenic VP in seafood at harvest. The effect of post-harvest handling and processing, and the ability to multiply to infective dose at the time of consumption.

We have divided our risk assessment into three modules: the pre-harvest/harvest module, the post-harvest, and the public health module.

The pre-harvest.harvest module will be presented by Andy DePaola -- I'm sorry, by Chuck Kaysner. Sorry, Chuck. It will include the shellfish water conditions, which is temperature, pH, salinity, nutrient profiles. All these are parameters that we've identified to be used in our risk assessment. The prevalence and levels of pathogenic VP in the water and in the oysters.

The post-harvest module will be presented by Andy DePaola. He will give more detail as to what happens post-harvest. The whole process. The post-harvest will include transportation, processing, distribution, storage, and retail. It will look at the prevalence and levels of pathogenic VP in the oysters, the handling and processing practices, the characteristics of growth, and intervention strategies. I know Bill Watkins went into some of the intervention strategies. Andy will provide some of the data.

The public health module we have divided into three sections; the epidemiology, consumptions, and dose-response.

Dr. Marianne Ross will be presenting most of the epidemiology from peer-reviewed literature.

Dr. Nick Daniels from the CDC will be presenting his data on the Galveston Bay outbreaks and some of the case series data that he has.

Mike DiNovi will be talking consumption, and Dr. Donald Burr on the dose-response.

In the public health module we will be looking at the number of vibrio parahaemolyticus infections that we know of, the level of pathogenic vibrio parahaemolyticus at consumption, case series data, as I mentioned, the number of normal gastrointestinal symptoms, the number of cases with septicemia, and I'm just talking about ingestion cases, and the probability of illness at different dose levels of vibrio parahaemolyticus.

Can we go ahead and start with the pre-harvest stage?

DR. MICHAEL JAHNCKE: We'll take a break for twenty minutes. We will assemble again at 9:30.

(Whereupon, a recess was had in this matter.)

DR. MICHAEL JAHNCKE: Yes, if we all take our seats we will get started again. Before we do, I just want to go over procedures a little bit, to reiterate. We will have time for public comment on the presentations. That will occur if we have some time this morning. According to the schedule our lunch is at noon. We're about twenty minutes ahead. If we have a little extra time this morning we will entertain public comments, and also at 1:00 o'clock this afternoon, it's scheduled from 1:00 to 1:40 for public comments. As Morrie had indicated this is a chance at this current time for the subcommittee members from NACMCF to ask questions of the speaker. Then it's also an opportunity for the NACMCF members who are observers in the audience to ask questions.

For those of you in the public, for the public comments, I do want to remind you at the front desk there is a sign-up sheet that indicates you identify yourself and the organization for asking public comments. So that is out front, the sign-up sheet up front.

With that I would like to introduce our next speaker, Dr. Charles Kaysner. Did I pronounce that right? He's going to be speaking on pre-harvest and the harvest module.

DR. CHARLES KAYSNER: Thank you. It's actually Chuck. Charles is my dad, so I go by Chuck just to keep us apart.

This is the team of the pre-harvest/harvest. I'd like to thank these particular individuals within the agency for helping to put the information together for this particular module. Marleen Wekell and Walter Hill are in my laboratory out in the Seattle area. Elisa Elliot and Brett Podoski from the Center For Food Safety, and Atin Datta from the Office of Regional Affairs in Washington, D.C.

This is what Fugeno (phonetic) saw back in 1950 upon first identification of vibrio parahaemolyticus. Bill mentioned that this morning. We have known about the organism for just about 50 years. There's supposed to be a big celebration in Japan next year.

I first saw this about 30 years ago now on a wet mount. This is sort of the family portrait of vibrio parahaemolyticus. As Bill Watkins mentioned this morning, it's very distinctive, a polar flagella. It's distinctive for the genes; vibrio cholera, vibrio alginolyticus, vulnificus and the whole batch of them all look like this. So using a microscope we can't really tell one from another.

Bill also mentioned that the majority of the environmental strains are non-virulent, or at least from historical data that we have that's what we suspect. He mentioned that they all produce the thermolabile hemolysin, which is species-specific. We are using this as a means of identifying the organism. I would like to see it designated as TL for the gene itself. There is a TLH gene that has been isolated from Drosophila, the fruit fly. So I think in the literature, or when you look into the gene bank system if you call up TLH you get all kinds of patterns for a Drosophila, which is a little bit different than what I want to work with in the laboratory.

Bill also mentioned the Kanagawa positive strains. The thermostable direct hemolysin is one of our virulence markers for strains environmentally and from patients.

Some strains also produce the thermostable related hemolysin. This was first identified in 1987 from a small outbreak in some Asian countries. It doesn't seem to be too prevalent as far as the number of illnesses. We're not too sure what the frequency of these organisms is environmentally. One of the things we did note, now that we know the sequences of these two hemolysin genes, when we went back and looked at our collection from the west coast, from patients and environmental strains, most of them produce both of these hemolysins. Now, whether a combination of these two helps to increase the virulence of these particular strains, we're not sure.

Bill also mentioned that historically when you look at the data that was published back in 1968, that most of the clinical isolates do produce the TDH, or are termed Kanagawa positive. And, when you look environmentally or in the seafoods that are on the market in Japan, a very low number were Kanagawa positive, one to two percent.

Now, overall, since our methods are a lot better now it would probably see a higher percentage environmentally and in foods. But, at least traditionally this is kind of in the picture that we've looked at.

In the U.S. I have the distinct feeling that the Kanagawa hemolysin is the primary virulence marker that we can use. When you look at the clinical strains from the various outbreaks in the country they're all Kanagawa phenomenon positive. This dates back to the Maryland outbreak in 1972, which was the first big one here in this country, and that was caused from boiled crab, and then with the oyster-associated outbreak on the west coast in 1997, and Texas and New York in 1998. They're all Kanagawa positive or TDH gene containing.

The historical data, when you look back in the literature, and this goes back to the late sixties through about the mid seventies, when you see some of the reports, the organism was first isolated in the U.S. in 1968 in Puget Sound out in our area by Barris (phonetic) and Listus (phonetic). So we didn't really get too active or too concerned about it until it showed up out our way, and then of course, the first outbreak then occurred in 1972 in Maryland. Whether it's been in this country for a number of years, probably has, or was it brought over by the Japanese ships, which were blamed at that time, that they deposited it over here.

But, most of the data that we found in the literature, virulence was not determined. We really didn't know how to determine virulence. We heard of the Kanagawa phenomenon and it is a somewhat difficult test for most laboratories to prepare. You do have to have fresh blood. The Food and Drug Administration sort of relied on our Taft Center at Cincinnati prior to its close because they had an old sheep out back and they could run out and draw some blood and prepare some plates and look at isolates.

The problem was it meant we had to ship the isolates back to Cincinnati, and then you're looking at three days to a week to get any kind of a result. And of course, when you're in the middle of an outbreak that's not really what you want.

The gene tests now are really helpful. We can do it right in our own laboratories. They are a lot simpler and easier.

When we looked at some of these studies then most of them showed that the levels of virulent strains were less than the total population of vibrio parahaemolyticus, if there was any data that was presented at all in that particular paper.

So, we feel that the older methods may have underestimated or missed the virulent strains. As Bill Watkins explained this morning, the problems in using the MPN system or an enrichment system is where the total population may have overgrown and massed over the levels of the virulent strains within the samples we're looking at.

Some of the things that we were looking at then is parameters for vibrio parahaemolyticus in shellfish. What's the routes of parahaemolyticus into the growing areas? I think we're pretty convinced that they have been here for a long time. They are naturally occurring, as Bill mentioned. How do they get into the shellfish? The prevalence of the virulent parahaemolyticus within the growing areas and in shellfish in relationship say to the total population with the non-pathogenic strains, the virulence of environmental vibrio parahaemolyticus, are they different than what we're seeing in patients or is there something that triggers these organisms or strains as they pass through the patient?

Then what kind of strategies can we use to prevent some of this? Are there some parameters that we can use to predict other than seasonality when we should maybe limit harvest from particular areas.

Bill mentioned they are naturally occurring. You can find them just about anywhere. Fish, birds, and animals probably help to move these organisms around in the estuaries. They have been isolated from fish. There was a publication years ago of the chance of birds in their excretion depositing the real parahaemolyticus from one area to another down the Gulf Coast.

Of course, the ship ballast is a theory of transport of various pathogens around the world. Vibrio cholera from South America to the Gulf Coast area was suspected to come up in ship ballasts.

The O3:K6 strain that showed up in Galveston Bay, they thought it came over from Asian area. It might well have. The thing that bothers me or scares me is, if it's in Galveston Bay and was also carried up to New York by ship ballast, it's on the west coast, we just haven't found it yet.

A study done quite a long time ago now, huh, Bill? Looking at levels of vibrio parahaemolyticus in the water in Narragansett Bay, where they found an indirect effect of the sewage discharge. Like Bill mentioned, these organisms are not indexed by human sewage.

However, they did find though, where there was higher incidents of sewage discharge, more nutrients in the water and higher levels of the organism, which sort of makes sense. They got some nitrogen sources and other things it might need to grow. And also, the zooplankton that they seem to be associated with could be in higher numbers because of the nutrients that are put into the area.

One of the things we have thought about, I don't think it occurs very often, is what we call relaying. This is where shellfish can be moved from one growing area to another, maybe to a cleaner water to purge the shellfish of some type of bacteria or virus or nutrients or what have you. I don't think this is a big problem with vibrio parahaemolyticus, since they are naturally occurring. Although in some respects we have some embayments, at least out on the west coast, that seem to have a high incidence of virulent strains. So there could be that possibility of moving in a relaying situation. If you went from one bay to another you might help to move these particular strains to a different growing area.

I put "contaminated" in quotes because, as Bill said, they're naturally occurring and they -- oysters are not contaminated, they've already been there.

I think one of the critical factors, and Bill's mentioned this, is warmer temperatures. This is a summertime organism. We usually have our outbreaks then. It's not a good time for microbiologists to take vacations during the summer because we are always in the laboratory looking for various organisms that are causing seasonal outbreaks.

Bill talked about moderate salinities. We don't see these organisms in real low salinity areas, or you don't see them open ocean. Besides the higher salinities, I think we have some pressure differences in some of these depth areas where the organism just will not survive.

Of course there is the association with the particulates and the zooplankton. Bill mentioned that they do break down chitin.

Dissolved oxygen I'm sure has some effects. Tidal flushing is something that we have looked at in the Pacific Northwest. A lot of the harvesters there will harvest their shellfish at extreme low tides. We can have some twelve-foot tide changes. During the summertime these extreme low tides occur right in the daytime, during our warmer time of the year, where you do get exposure, ambient exposure by the shellfish to some quite significantly warm temperatures compared to the water temperature that they're normally grown in.

There will be a study initiated, I believe, or maybe it's even actually started, that is funded by the ISSC at the University of Washington. They'll look at the effects of some of this. We think that this is one area where we might be getting some increased counts due to the warmer temperatures that might be occurred by the oyster as they sit in the open environment.

We mentioned a little bit about phages and Dr. Eklund's question. I think he's on to something here, at least environmentally. We know there's some phages out there. When you get them in the laboratory, if you bring a sample in that has parahaemolyticus you have probably brought in the phages. They might do right well in our own little enrichment systems to help lyse those particular organisms we're looking for, giving us lower counts.

There's also the inner-cellular parasite Bdellovibrios. There's been a number of studies done with this. That's a seasonal occurring organism also, which makes sense since they prey on vibrio parahaemolyticus they're going to be around during the summertime when parahaemolyticus is more prevalent.

Bill's covered some of this already. Generally we're looking, the water gets 15 degrees Centigrade or above we start seeing vibrio parahaemolyticus. We do see the over-wintering in the sediment layer, particularly out on the West Coast in the nice silty sediments that we have, it's the best time to go looking. So here's where some of our sample strategies are, during the wintertime it's best to look in the sediment if you just want to find the organism. We do not see it in the oysters much at all during those periods of time, or in the water.

Bill mentioned the viable non-cultural state. I know this is under quite a bit of debate. A number of gram negative organisms have been shown to go into this survival strategy at nutrient deprivation, and also at low temperature. Vibrio cholera, vibrio vulnificus have been studied. Nobody has really looked at vibrio parahaemolyticus. It might be true that we see this type of thing occurring. What it means environmentally could be that maybe that's one of the reasons during certain times of the year, or in certain environments, that we can't find the organism.

Again, we've talked a little bit already about the planktonic species and the association of the organism with those.

One thing for sure, it's a seasonal occurrence. Bill's mentioned this, and we see a seasonal occurrence in the environment. This, at least in the U.S., was first published by Kaneko and Colwell in 1974, and there have been a number of other studies that show the same thing.

Seasonal occurrence in the environment. Then they're also seasonally in the shellfish and also we have seasonal occurrence of the illnesses.

This is a slide from Tilton and Ryan back in the late eighties. I use it a lot because it's nicely depicted to show that a temperature of 15 degrees to 22 degrees in the water the vibrio parahaemolyticus counts go up quite dramatically, July, August. September, they start to trail off. A very typical pattern that we see for vibrio parahaemolyticus, vibrio cholera, vibrio alginolyticus, you name it.

When we see more of them in the environment we see more cases of illness. This is Washington State data for the last eight or nine years, and if you look at July and August, over 80 percent of the illnesses occur in those two months. I have highlighted three of the years that were actually termed El Nino years. 1990 was also a warm summer, at least up in the Pacific Northwest. Although I don't think it was really designated El Nino, but, I definitely think that this is one thing that is occurring to warm up the temperatures and also increase the frequency of vibrio parahaemolyticus in our area, and probably others. And also, increase the number of cases.

Bill's talked about some of the methods that we used previously. The MPN being one of them. We have had some real problems with trying to isolate the virulent strains from the total population with the MPN system.

Your probability of picking off a plate after you enrich these organisms is pretty slim. Can we really compare the data from some of the previous studies that have been published? I can think of at least four different enrichment routes that have been used for environmental studies for vibrio parahaemolyticus, most of them have been developed in Japan, and they have used antibiotics and various things to help select for the species itself. So maybe you can't compare the counts because of the differences environmentally. But, at least we can show seasonality. All of these studies show that same trend.

Bill has mentioned the direct plating gene probe technology that we're coming up with. Things are looking pretty good. There are a number of training sessions going on, as mentioned previously.

How do they get into the shellfish? Well, it's been mentioned, and you all know, that oysters, clams, et cetera, are filter feeders. So if they're in the water, pretty much they're going to end up in the shellfish. I have to say an oyster is a pretty amazing animal. I can put him in a sink in some artificial sea water and dump in any kind of organism I want to look at, and within four hours I can get enough uptake of that particular organism to do any kind of study. It's just amazing what these animals will do.

What is the prevalence of vibrio parahaemolyticus in shellfish growing areas and in shellfish? We have seen, on the West Coast anyway, a few areas where we have a higher incidence of illnesses reported, particularly in the last two years. Quilcene Bay, which is a small embayment off Hood Canal and the big Puget Sound Basin, has been responsible for quite a number of illnesses in 1997, and a significant portion of the illnesses we had last summer.

There's a couple other areas that have been responsible for illnesses also back in the early nineties that are also in the Puget Sound Basin. So we think we have some particular embayments that the virulent strains that we're looking for seem to be more prevalent.

Can we develop some predicative models? I think the El Nino/La Nina patterns are something that we can really start to key on. We are in a La Nina year this year. I'm curious to see what happens this summer. Water temperatures, air temperatures have been very cold out in the Pacific Northwest. Some day we might even have a Spring out there. We did have a couple nice days right before I left town. But, it's been quite cold there. The water temperature has been cold.

During the 1997 summer one of the oyster growers, a major grower down in Willapa Bay, which is in southwest Washington coast, they monitor temperatures in his growing area and entrance of the sea water, ocean water into the embayment, found it an average of four degrees Fahrenheit warmer during the summer of 1997, an El Nino year, than what he recorded previously. And also in his growing areas, four to six degrees Fahrenheit warmer.

Now, it doesn't sound like a whole lot of temperature difference to us, but maybe to a vibrio parahaemolyticus or even to an oyster it can be quite significant.

So temperature, I think, is the main thing we're looking at, and what we have to look at.

This is some temperature data from a study we did back about twenty years ago. This is one of those bays where the tide goes out and harvest occurs on foot in the growing area. We tried to plot temperatures seasonally. One thing with the water in the Pacific Northwest it stays relatively cold all year around. Kids will swim in it. Us older people, it's just too darn cold. Pretty consistent. During the summertime it might get up to 60 degrees on the surface temperature. But, these are, at least on high tide temperature, these mostly overtake about three feet off the floor above the shellfish areas, and we did have quite some tidal differences, up to eight, nine feet difference.

Low tide temperatures were usually from a puddle next to the oysters that we were going to collect for our analysis. So we can see during the summertime, at least out there, we get some pretty warm temperatures, but it's nothing like the Gulf Coast is getting.

This is some of the data from the samples that we ran. Seasonal appearance, particularly in the oysters, July, August, September is when we found the levels to increase. But, if you look at this, there are quite low numbers. This is a log scale of the levels that we did find, and even during the summer. They're averages. We would have a few samples that would -- are we going to self-destruct here?

(Pause.)

MR. MEL EKLUND: Chuck, while we're waiting, could I ask you a question? This is Mel Eklund. The water temperature there is this a low tide or a high tide water temperature?

DR. CHARLES KAYSNER: On the slide previous?

MR. MEL EKLUND: Yes.

DR. CHARLES KAYSNER: There was low --

MR. MEL EKLUND: (interrupting) This one here, you have water. I think that's -- what does that refer to?

DR. CHARLES KAYSNER: Oh, this is count per gram, count per hundred gram. Log per hundred gram of water, sediment, and oyster samples taken during that same seasonal study that we did.

The line doesn't come out, we're looking at August, the sediment we had a log of two, which is what, a hundred grams. Oh, actually that should be per gram, because I did convert that out. But, generally the count is very low. Occasionally we would see an oyster might get up to 1000 total vibrio parahaemolyticus. They were very seldom higher than that.

DR. MICHAEL JAHNCKE: Go ahead.

DR. CHARLES KAYSNER: Okay.

DR. ROBERT BUCHANAN: Could you go back to that slide for a second?

DR. MICHAEL JAHNCKE: Go back one slide.

DR. ROBERT BUCHANAN: For those where there's nothing, you found nothing in the water.

DR. CHARLES KAYSNER: Correct.

DR. ROBERT BUCHANAN: Okay. You indicated that oysters were really quite a remarkable animal because of the way they can concentrate the organisms within themselves. However, I go over to -- I'm trying to get a feel for the level of concentration. So August you're getting about a tenfold concentration of vibrio.

DR. CHARLES KAYSNER: Looks like it, uh-huh.

DR. ROBERT BUCHANAN: If you move over to September this concentration effect isn't there. In fact you have what, two orders of magnitude less in the oyster than you have within the water table, the water column. What factors influence the concentration effect?

DR. CHARLES KAYSNER: Definitely the filter -- or the feeding ability of the oyster. This is one thing we've looked at in other studies that if you have two oysters laying side-by-side we can have some real differences in count if you look at them individually. This is data that in some of these months is probably like ten different daily oyster samples, high tide then low tides. So we're looking at like twenty samples during a period of time for some of these months. Others were just a lesser number of samples collected, so the data is a little bit skewed that way. This is a total of high tide and low tide, so the counts are more of the average, where generally what we saw was at the low tide sampling, and this is where they were exposed at least during the summertime, the levels in the oyster were higher than during the high tide. After they would come in they were maybe able to purge some of the vibrio out of the oyster itself, after the tide has been in.

I have another slide here that will show a little bit more on the relationship between water and shellfish, from Andy's laboratory.

DR. ROBERT BUCHANAN: So you're saying that the concentration goes up when the organism is at low tide, and exposed they're not actively filtering at that point.

DR. CHARLES KAYSNER: No.

DR. ROBERT BUCHANAN: Is the microorganism actively growing within the oyster at that point?

DR. CHARLES KAYSNER: Don't have that data, sir. That's something we would like to look at. The study that was just initiated at the University of Washington, I think, is going to address this type of approach.

This is -- I kind of put together some studies that were done at the three different coast lines. Basically, you know, when you look back at some of this historical data generally the levels that they found were quite low. The methodologies, of course, have been all somewhat different. The Watkins and Cabelli study was done by membrane filtration. Since they had water it's a nice easy way to look at water samples. Some of the others have used MPN's, but generally what we see, on the east coast the water temperature is very similar.

These studies are the west coast. This is a lot of data from our laboratory. On the presence and even levels, what we have generally seen is the levels do not get very high in the samples as we take them in our studies, where we're going out and doing our own sampling.

Occasionally, you will see some oysters with, at least in one of the studies, up to about 10,000 per gram environmentally. But, that seemed to be very rare that we did get that kind of an occurrence. Water samples up to 1,000, maybe. A pretty high level that we're finding in the estuaries that we looked at in these studies.

Again, the temperature is very similar that we saw on the east coast and west coast. But, when you go to the Gulf Coast water temperature is quite dramatically different. I mean, that's the hot tub down there. The thing I've always been curious about the strains of vibrio parahaemolyticus we see in the Gulf Coast are adapted to a warmer temperature versus the ones, say on the west coast.

Where we see difference is, environmentally if these strains were transposed to different areas. I think you're going to see some different growth response with some of these organisms, depending on how you culture them previous to the work you're going to be doing.

But, generally this is what we've seen. Basically low counts overall, occasionally a high oyster, but not that often.

So what is going on then with the illnesses and what's causing the illnesses that we're seeing with some of the outbreaks we've had in the last couple years?

What are some of the best sampling strategies? As I mentioned, if you look at an individual oyster very differences in count. One can have ten per gram, the other one can have 10,000. If you eat twelve oysters in a sitting, what if you got the hot one? But generally, when you put those oysters together and make a composite, the overall count is low. Maybe we're missing something here.

The relationship of vibrio parahaemolyticus and the water column to the sediment and shellfish, this, I hope, will answer your question, Dr. Buchanan, we see a change in the seasons, of course. If you're going to sample, or look for vibrio parahaemolyticus in the wintertime the best thing to do is look at the sediment.

This is some data from Andy DePaola's laboratory, where if you looked at the levels of the overlying water to the shellfish at that sampling you see ten to a hundredfold difference in levels in the shellfish than the water. Possibly this could be used as one of our predictors of -- in a particular growing area if we keyed in on the water samples. When they reach a certain level maybe we say, this is time to limit harvest due to the possibility of high levels of vibrio parahaemolyticus.

Again, this is going to be based on temperature, of course, too. But, it gives us something, and at least in the laboratory it's easier to analyze a water sample than to shuck a dozen oysters and blend them up. You have a lot of other factors in the oyster too that tend to -- at least from what we've seen, from pH changes in the glycogen that we discussed a little earlier, sometimes we're getting false counts because of those things within the oyster just destroying the bacteria you're looking for after you make a milkshake out of that particular sample.

Strain differences we have discussed. These will be the various strains that produce the different hemolysins.

Shellfish species factors. We think this has something to do with it. It was brought up earlier about the amount of glycogen within the oysters. What about during spawning, at least out in the northern regions? The Pacific oyster spawns during the summertime, and there is a lower glycogen level. I've always thought that maybe at this time the oyster is more susceptible to uptake of vibrio organisms, not just parahaemolyticus. Although during the 1997 outbreak, from what one of the oyster growers tell me, they have a triploid oyster that was bred so that it won't spawn. So you have a nice oyster during the summertime. Those cause illness too.

So some of the answers lie within the shellfish itself. We're not sure of, what makes them more susceptible. Are there some chemicals that are getting into the water such as the creosotes from docks, and wood preservative is something that might be effecting the oyster, making them a little more susceptible to some of the organisms at the uptake.

The last item, there is something just published early this year by Iida and co-workers in Japan. They have looked at a number of strains that have caused illness in Japan and in other Asian countries that contain both the TDH and TRH genes. All these organisms are urease positive. These are the strains that we see on the west coast. They've determined that the urease gene and the two hemolysin genes are all within a certain portion of the chromosome within these organisms.

They don't call it a pathogenicity island, but recently what was published with the vibrio cholera epidemic strains is, all the genes that regulate cholera toxin production and virulence and everything else involved in classical cholera, are in a pathogenicity island, which has some chance then of being more readily transferred environmentally by phages, that Dr. Eklund brought up. Maybe we're seeing some movement of these particular virulence mechanisms among strains by phages.

But, there are some publications for various other organisms where you can actually move a particular type of toxin from one organism and one species to another within bacterium.

So, possibly we're seeing something like this.

This is some of the information that we got after the peak of the outbreak in 1997 on the west coast, where the poor State of Washington was up to their eyeballs in samples and we tried to help out in our laboratory, doing some of them. Again, we were past the major peak of the outbreak. The industry had voluntarily stopped harvesting and distributing. We were able to obtain some samples both from the industry and from the state to look at. The levels were quite low overall. We had one sample up at 46,000 per gram. Within that sample we found no Kanagawa positive strains.

Of the samples that we did find you can see the values, 3 and 7.3 MPN per gram within the two samples that we found. Not real high numbers. So what is it that's causing illness if indeed this was reflective of what was going on during the outbreak?

The seral groups we're seeing, Bill mentioned this earlier, the 04 is a big illness producer on the west coast. This is the one we see primarily. There are some 01's, during 1997. I have some patient strains from the Washington Department of Health. Most of them are 04's and a few of the 01's, both Kanagawa positive. Both urease positive. Very consistent from what we've seen over the last twenty years out on the west coast.

The State of Washington went into some monitoring after the outbreak and looked at oysters during the season here. Had one strange sample that came up from an area up in the San Juan area, it was greater than 110,000 MPN. They're not really sure, something might have happened to that sample. It was sent down from up north down to the state lab. But generally, the overall samples through the year were low and of course during the wintertime they do not find very much at all.

Data that Andy sent up from -- came out of Galveston Bay after we got involved looking at samples and the lyses down there. Again, after the peak of the outbreak. The one thing with this is this is all data generated using the gene probe techniques that we have started. Counts quite low overall. We didn't find the 03:K6. We did find two samples that had Kanagawa positive strains. One of them was, I believe, serotyped by CDC, which was an 08, which is completely different than what we've seen on the west coast and was completely different than the 03.

But generally, the counts were low. I think the thing I'd like to see here though is the water temperature. You look at the temperatures in Galveston Bay, water temperatures on the west coast don't even reach what these temperatures are. So, we're looking at a different environmental system altogether here.

Can we determine if all virulent strains are equally detectable? We're getting some nice methodology now, I think, with the gene probes for the TDH. But, are there other factors with these organisms that might also have to be there to cause illness? Some strains have produced shiga toxin-like toxins. Some produce some true enterotoxins very similar to the e-coli organism, and there are some other things. But, there's probably a number of things that are required by these organisms to cause illness besides the TDH. But, at least with some of the work that's been done, if you excise the TDH gene out of vibrio parahaemolyticus you could not get fluid accumulation in a rabbit ilia loop, which used to be the test that they -- for the animal model to demonstrate that.

One of the things we should really look at, and maybe our new probe procedures will help, is to determine the relationship of the virulent parahaemolyticus within the environment in relation to the total number. The probes now seem to be working quite well and this might give us that data that we need.

This was brought up a little earlier by Bill. The ISSC is funding some monitoring studies by the states, as Andy mentioned. Some training is already ongoing. They're gearing up to get started. It is going to involve the three coasts. This might be the method sensitivity that we need to look environmentally to predict the number of Kanagawa positive strains that we see in these various areas. Then as this data is put together, which unfortunately, Dr. Buchanan, won't be until after July, since July seems to be when these organisms start to show up, take a look at this 10,000 per gram level that we've used for raw shellfish. It might be that we can set that at a different level to give the state something to use to monitor their bay and maybe take some closure actions.

Strain differences. We talked a little bit about, at least on the west coast, you know, the urease positive ones are big, the Texas strain or the Calcutta strain or the 03:K6 distinctly different is urease negative. And, it does not produce TRH. So it is a distinctly different strain than we see on the west coast. So at least if we start seeing some illnesses from something other than what we're generally looking for, we can start maybe keying in on maybe we do have the 03:K6 on the west coast.

The one thing nice about the urease enzyme is it's quite an easy test to test for in the laboratory, and you can use it quite nicely as a screening procedure with the isolates you get to look for urease positive, and then concentrate on those, because that's where your virulent strains will be.

We talked a little bit about some of the shellfish species factors that may influence the environmental virulence of vibrio parahaemolyticus.

Some of our strategies. How do we prevent shellfish from taking up virulence strains? If they're there environmentally they're probably going to get into the shellfish. How do we develop strategies so contaminated shellfish are not harvested? Well, maybe with our new methodologies this is something we can look to, to help the states address this that when things reach certain levels, whether it be in the water or in the oyster, that this would be the time to look for some closure of harvest of those particular areas.

One of the things we know for sure, they're seasonal trends. It's pretty obvious from all the data that we've looked at. Geographical areas are prone to virulent strains. As I mentioned before, Quilcene Bay in Washington, Galveston Bay in Texas.

High attack rate. The 03:K6 seems to be a strain that could cause a significantly higher attack rate than we've seen with some of the other strains of vibrio parahaemolyticus. So maybe then with our newer probe procedures and detection maybe we should just concentrate on the TDH producing strains versus just the general population. It would sure be easier in the laboratories for the laboratory worker to only be concerned about one particular aspect of the organism.

I believe that's it.

DR. MICHAEL JAHNCKE: Before we have questions from the subcommittee, I want to remind everyone to identify themselves and their organization. Any questions from the subcommittee group? Cathy?

MS. CATHERINE DONNELLY: Cathy Donnelly. Chuck, the temperature and what's going on with respect to climate, we're obviously getting warmer with the temperature and presumably that has some kind of impact, but what about colder temp -- is there a temperature for die-off of the organism, or are you looking at whether the coldest temperatures have risen in factoring that into a risk assessment model?

DR. CHARLES KAYSNER: I haven't really looked at where the cutoff point is. It seems 15 degrees environmentally seems to be one of the triggers. Bill mentioned some of these strains will grow at lower temperatures in the laboratory. But, there you have a system, you know, you're sort of helping them by putting things in there. Environmentally we really haven't got that data to show that say at 14 degrees that we don't get some slow growth. But, you're right. I think the water temperature itself, at least in the northern reaches of the U.S., 10 degrees, I think, is a good cutoff temperature that we are not going to see the oysters -- environmentally you would only find it in the sediment and not in the oysters in the water.

MS. CATHERINE DONNELLY: The nature of my question is, with milder winters maybe we're just not getting enough time in that die-off temperature and that's a factor in promoting --

DR. CHARLES KAYSNER: Right. There is an individual from the Meteorological Department, University of Washington, who is looking at these El Nino patterns and trying to work with the State of Washington as far as the illnesses that have occurred. He's got some nice big satellite pictures of the warm trends in the waters and the currents in the Pacific Ocean that we've seen for the last two years. I just recently saw, about two months ago, saw a slide of what they've got right now for this La Nina year and it's quite significantly different. So it will be interesting to see what happens this summer.

With more awareness of what's going on, at least on the west coast, there's a lot of people that are tracking water temperatures a lot more, so this might give us some information.

DR. MICHAEL JAHNCKE: Bob?

DR. ROBERT BUCHANAN: Yeah, Chuck, I have a series of questions as I'm trying to go through an assessment of what factors you might have to deal with in this section of the module. First, just to help me along, you've indicated that approximately 98 percent of the cases are associated with TDH positive VP's. How confident are you about the percentage that are non TDH positive? Is that a constant percentage or is that something that you're unsure of?

DR. CHARLES KAYSNER: Is that clinical or environmental?

DR. ROBERT BUCHANAN: Clinical.

DR. CHARLES KAYSNER: Clinical?

DR. ROBERT BUCHANAN: Of the clinical cases you see you've indicated that approximately two percent are TDH negative. Does that reflect -- what does that reflect? Should we worry about that?

DR. CHARLES KAYSNER: On the slide I had earlier?

DR. ROBERT BUCHANAN: Yeah.

DR. CHARLES KAYSNER: That was actually environmental and seafood isolates. What the Japanese had reported was one to two percent.

DR. ROBERT BUCHANAN: What's the percentage of TDH negative strains that have been implicated in outbreaks, in sporadic cases?

DR. CHARLES KAYSNER: I have one strain from Idaho Health Department from about ten years ago that they said was from a patient who ate oysters. It does not do anything. I am wondering regarding that particular strain. Was that the organism that caused the illness or were they looking for something else.

I think we're going to see, maybe not in this country, but in the Pac Rim countries where we're seeing more of the TRH producing organisms, the thermostable related hemolysin, which is different, distinctly different, there are reports of illness from. That seems to be becoming more prevalent. We haven't seen it yet in this country. Eventually we will.

Now, there's some illnesses that have been reported in Vancouver, Canada area from non-Kanagawa positive strains. But, there's no data to show what else might be occurring, were there other toxins. But, we do not have an in-vitro test right now to demonstrate that TRH. It will not show up in the Wagatsuma agar, which has traditionally been used for the Kanagawa. So, we don't have a test. There is a Liza test in Japan. It's an expensive test to buy. I've never even wanted to try it because we don't see that many strains. We can do it genetically in our laboratory anyway. But, most of the patient strains we looked at have TDH. They might have the other one, but they have TDH.

DR. ROBERT BUCHANAN: Assuming that the evolutionary goal of vibrio parahaemolyticus is not to make humans sick, what is the function of TDH in the environment in which vibrio lives? Do we have any information about that?

DR. CHARLES KAYSNER: No. When I started graduate school 25 years ago, John Liston, my major professor said that I should do my dissertation on what triggers TDH and why is it necessary. Well, I'd still be in graduate school. Yes, we're not sure.

What advantage do these hemolysins have for the organism in the environment?

DR. ROBERT BUCHANAN: Urease, at least in urease species, has been associated with the acquisition of acid resistance. Is it similar within vibrio parahaemolyticus?

DR. CHARLES KAYSNER: It didn't say too much about it. I think Andy is going to address that in the next presentation. But, yes, there is a publication regarding that.

DR. ROBERT BUCHANAN: I would also, if at all possible, can we go back to the slide where you indicated the relationship between the water column and the concentration in the oysters. It was a nice pale blue background.

DR. CHARLES KAYSNER: Andy's data?

DR. ROBERT BUCHANAN: Yes.

(Pause.)

DR. ROBERT BUCHANAN: This graph shows pretty good what appears to be a linear relationship that you could use as a predictor at least of initial contamination. My only concern is over there on the left-hand side you have a couple of oysters there that apparently were placed in very low levels, but concentrated the organism five waters of magnitude.

DR. CHARLES KAYSNER: Right.

DR. ROBERT BUCHANAN: Do we have any explanation of why certain oysters seem to be much better at concentrating vibrio out of the water column than others?

DR. CHARLES KAYSNER: Andy, do you have anything you want to say about this?

DR. ROBERT BUCHANAN: Any potential for post-harvest growth in those samples?

DR. CHARLES KAYSNER: I think Andy will probably address that in our next module here.

DR. ROBERT BUCHANAN: Why the outlyers?

DR. MICHAEL JAHNCKE: Andy, if you could use the microphone, please.

DR. ANDY DEPAOLA: I would just say natural variability on the outlyers. But, I also want to mention that this is per hundred grams and not per grams. That's one of the reasons these counts seem so high, and these samples were collected by state people and shipped on ice. We analyzed them within 24 hours. I'm afraid back in the eighties we were less aware of the post-harvest growth of vibrio parahaemolyticus and the temperature of the oysters on receipt may not have been as well controlled as what we're currently doing.

DR. ROBERT BUCHANAN: So we have a fair degree of uncertainty about the relationship that you've depicted here.

DR. CHARLES KAYSNER: At least with some. But, I think generally when we look at some of the studies that have been done, and even from our laboratory, that the shellfish seem to have ten to a hundredfold more than the water if you collect that overlying water. It's kind of a general pattern. Do we need some more data then to maybe crunch to see if that gives that nice linear pattern. But, maybe it could be used as a trigger.

DR. ROBERT BUCHANAN: I was just trying to think, you know, this would make a very nice relationship in terms of a risk assessment except for your outlyers.

DR. CHARLES KAYSNER: Except for the outlyers, right.

DR. MICHAEL JAHNCKE: Mel?

MR. MEL EKLUND: It's Mel Eklund. Chuck, I think you brought out a couple of very interesting factors here. One is the enumeration procedures. Remember in our 1997 meeting following the outbreak there, there was a tremendous variation in the vibrio counts after the outbreaks. Some of them were very, very low.

I think you made a very interesting point though, that the oyster side-by-side can vary as much as a thousand, ten-thousand-fold. I think this is a great part of this whole problem we have here, is in evaluating in a risk assessment.

The valuation of 10,000 organisms per gram, I know in the many meetings that I've attended in Japan, when they have discussed vibrio parahaemolyticus, I know Dr. Sagasaki (phonetic) often mentioned that once the organisms reach the level of approximately 100 to 1,000 per gram he was very concerned because of the rapid generation time of the organism.

The other thing that I wanted to mention is that you were talking about the spawning or the sexual maturity of the oysters. I'm not sure how oysters respond, but I know in fin fish, as they approach sexual maturity their approximate analysis changes dramatically. Their protein levels decrease. The protease, enzymes increase considerably, and all of these may play a factor of the susceptibility of the oyster itself as to invasion by the organism.

You made some good points on that.

DR. MICHAEL JAHNCKE: Bob?

DR. ROBERT BUCHANAN: Another follow-up question I'm trying to assess factors. You have some oysters that live forever underwater, depending upon the depth of the bed and the tidal situation, and some that are periodically, through low tide, exposed to the air. Is there any differential that needs to be considered in terms of incidents and prevalence of vibrio parahaemolyticus in these two types of shellfish?

DR. CHARLES KAYSNER: I'm not sure of all the data from the illnesses on the west coast the last two years. Quilcene Bay, of course we mentioned it, had the higher incidents of illness. So there's something about that particular estuary. But, I'm not sure of the harvest technique in that particular area. This is one thing I wanted to check with the industry on. I believe it's a quite shallow area, and the temperatures are probably quite a bit warmer than say other areas. But, whether you actually get the exposure there in those growing areas, I'm not sure. Because, I'm not sure where the lyses are within that part of the estuary.

But, we did get illnesses from harvest areas where you don't have that exposure. So it had to be that probably the counts or the infective dose, what have you, was obtained by the oyster from the water at that particular time, and probably the temperature of the water was quite significant in that.

DR. MICHAEL JAHNCKE: Are there any other questions?

Let me ask you just one and then we'll move on to Dr. DePaola. In Quilcene Bay you indicated that the incidents of virulent strains is much higher. Is there anything unusual -- you mentioned a few things, unusual about that bay or has that area been used more for relaying areas, or is there any balance water differences than in other areas?

DR. CHARLES KAYSNER: I'm not sure. In fact, Robin Downey is here from Pacific Coast Oyster Growers. I might be able to say a little bit more about Quilcene Bay. I mean, I've been there and done some sampling, but generally I'm not sure of all the things that go on there. Do you have any information, Robin, on something --

DR. MICHAEL JAHNCKE: Maybe could hold that for the public questions.

Dr. Kaysner, thank you very much for your presentation.

Our next presenter is Dr. Andy DePaola. He's going to be speaking on the post-harvest module.

DR. ANDY DEPAOLA: Good morning, committee members and members of the public who are stakeholders in the shellfish safety issue, and as was mentioned, I will be doing the post-harvest section.

I've had a lot of help with this, particularly from our laboratory, the Gulf Coast Seafood Laboratory, and from the Office of Seafood and Division of NMF.

Many of you are familiar with oyster processing techniques, but maybe some of you aren't, and I'll briefly review what happens with oysters after their harvest, until they're consumed in this flow chart.

Oysters, unlike most animal products, are not slaughtered at harvest. In fact, they are generally kept alive until consumption. They do quite well for days at ambient outside temperatures and can live for weeks when refrigerated.

The harvest varies according to the geographical area. There are a number of techniques. Hand-tonging is required in some states, such as Alabama and other states. Dredging is the most popular. And, as Chuck mentioned, sometimes in low tide oysters are hand-picked and placed in baskets and floated up on high tide and harvested. These may all have some impact on vibrio parahaemolyticus levels, perhaps the last one most of all.

When the oysters are placed on the boat, the next process is culling. This is simply knocking off any shells or small mollusk from the commercial oysters.

Then they are stored on board usually a small vessel, which is nearly always without refrigeration capabilities. The time that they may be stored could vary from just a few hours to more than a day, in some cases.

When the oysters are landed, they are usually loaded onto trucks. The requirement for refrigeration varies from state-to-state. If oysters cross state lines then it is federally mandated that these vehicles must be refrigerated.

There are two types of processes that go on. Mostly -- well, the oysters that are consumed raw are usually processed as shellstock. That is, live, in-shell oysters. This procedure is very simple. They are sprayed with water to wash off mud. Placed in cardboard boxes, and then they are transported to wholesalers or restaurants. They are maintained alive there until consumed.

As I mentioned, most oysters intended for raw consumption are processed this way.

On the right-hand side, this is the procedure for processing shellfish meats. They are shucked by hand, and the abductor muscle, which is connected to the top and bottom shell is severed. Then the next step is to wash the mud or loose shell fragments. This procedure is called blowing. It also tends to add water to the oyster meats and thus reducing the salinity somewhat.

They are packaged usually in metal or plastic containers, and these are stored on ice, generally, until they're consumed. These are generally intended for cooking, but in some cases they are eaten raw as shooters.

The meats, like I say, are normally kept on ice, whereas on the shellstock, those are kept from 45 to 50 degrees, except while they're being washed and boxed.

Really, there are only two questions that this segment addresses. The first is, the shellfish industry harvesting techniques, do they effect the vibrio parahaemolyticus risk or the risk of illness?

And secondly, are there handling and processing technologies that reduce the vibrio parahaemolyticus risk?

Don Burr will talk more about the dose-response. But generally, risk goes up as the numbers of organisms consumed goes up. So, I will focus primarily on densities of vibrio parahaemolyticus.

On the first question, whether the industry practices effect vibrio parahaemolyticus densities, our approach has been to compare the levels at consumption compared to harvest. I'd also like to clarify, when I say "industry practices" it is not specific for any one segment or any one practice. It goes from the harvester to the server at the restaurant.

Chuck has just reviewed some of the levels that have been observed in oysters before harvest, and as Bob asked, if we're certain of anything, I think the one thing that we're certain of is that there are mostly uncertainties when it comes to predicting the levels of vibrio parahaemolyticus in the environment, and particularly the distribution of pathogenic strains.

This is also a study that Chuck has shown the relationship between water levels and oyster levels. It was a nationwide, and what we found in sampling four times, representative of the various seasons, from various locations in each coast, that the highest counts that we found were along the Gulf Coast.

We screened 200 strains and only two were shown to be pathogenic.

This represents more intensive data that was collected following the outbreak of 03:K6 in Galveston Bay. The outbreak began in late May, and the oyster harvesting was terminated on June 26. Here we gathered our first sample late in June. This was a sample where we found our highest counts. The red bars there are the means and the yellow is the standard deviation.

This was the only time that we sampled and found greater than 10,000 per gram of the 106 samples that were collected. During the remainder of the study, levels remained fairly constant between 100 and 1,000 per gram through September of that year. Of these samples, it includes more data than what Chuck had shown, there were three samples in which TDH positive strains were detected of the 106 samples examined, and they were present at 10 per gram or higher.

To get this, we looked at thousands of isolates as we used direct plating. Whereas, in earlier studies we were only able to examine much fewer isolates.

Just in review, what we know most of is when vibrio parahaemolyticus are low, and that's in the wintertime. Winter is longer on the Pacific and Atlantic coasts than it is on the Gulf. When conditions are ripe for vibrio parahaemolyticus their numbers tend to vary quite a bit, and we know very little about the distribution of pathogenic strains.

Fortunately, because of a study that we started last June in cooperation with ISSC and the states, we have a lot better information on the levels of vibrio parahaemolyticus, and the study also included vibrio vulnificus in retail oysters. That was the primary purpose of this study, and the secondary purpose was to collect isolates of vibrio parahaemolyticus for further characterization.

There's been, like I said, a lot of assistance with this study including the ISSC, the various states, FDA laboratories and the Division of NMF, National Marine Fisheries. Thank all of you.

The red dots show places or states in which samples are collected twice a month, and the black shows the analytical laboratories, which are Denver, Dolphin Island, and Atlanta. Samples from the various states are rotated to these laboratories to reduce any laboratory bias. These states were selected because of their various geographical distribution and also states were selected because of a history of being associated with vibrio infections.

Through March this year we've analyzed 310 lots of shellfish. Most of them have been harvested on the Gulf Coast as this has been the area most commonly implicated with vibrio illnesses.

The dividing line between the mid and north Atlantic is the New Jersey/New York border. A similar number of isolate samples have been tested from the west coast and the Canadian samples have come from the Atlantic coast.

This shows a little bit where samples were collected and where they were harvested. What's highlighted here are states that are on the coast, and what is seen here is that the oysters consumed in those states are usually home-grown.

In order to get better representation we have strived to not sample the same establishments over and over. Out of the 310 lots they've come from 259 establishments. Most of these have been restaurants, as most raw oysters are consumed in restaurants, and most vibrio illnesses have been associated with restaurant oysters. Seafood markets and wholesalers were also sampled.

This is a rather busy slide, so I'll take a few minutes on this. What we see in Canada, west coast, and the North Atlantic coast is that the dark here is a high-level, undetectable, that's less than .2 per gram vibrio parahaemolyticus, and in these samples exceeding 100 per gram are not that common.

On the mid-Atlantic, on the Gulf Coast, we see quite a different picture, with about 10 percent of the mid-Atlantic and about 20 percent of the Gulf samples exceeding 10,000 per gram. This is a much higher frequency than what we saw at harvest in some of the earlier studies where you occasionally saw 10,000 per gram, but that was a very unusual situation.

This looks almost like the same slide, just substitute vibrio vulnificus. We see the distribution. A lot of non-detectables on the Canadian, west coast, northeast Atlantic. Once again, numbers exceeding 10,000 on both the mid-Atlantic and the Gulf Coast.

This is a summary. This only goes through January. What we see is vibrio parahaemolyticus is slightly higher on all coasts than vibrio vulnificus, except on the Gulf Coast where vibrio vulnificus is about five times higher than vibrio parahaemolyticus.

This is a summary of a study that was recently completed at the University of Florida, and Dr. Gary

Roderick (phonetic) has provided this information. There was also a retail study. Seven establishments were sampled each month from September through May, and vibrio parahaemolyticus was determined using the direct plating and DNA probe methods. There's a lot of similarity in this data and the current FDA retail data. You see in September, October, November we're getting the highest counts. About 10 to 20 percent of these samples exceeded 10,000 per gram. Whereas, December through May the mean counts were generally less than 100 per gram.

I think this data answers the question of whether the industry practices do effect vibrio parahaemolyticus densities. On the Gulf Coast in particular higher levels of about one to two logs were seen at consumption than they were at harvest. The data is not as abundant for the other coasts, and the harvest levels are not as well established. But, I think we would see probably the same sort of trends, but probably not as great, because the ambient temperatures are a little bit lower.

As has been mentioned earlier, temperature has been shown to be a major factor controlling vibrio growth and survival. I wanted to briefly review some of the parameters I think are needed for risk assessment. The first time is the lag time. That's the time before the organisms start growing exponentially. A doubling time is the time it takes for them to replicate or double at the time when they're growing at their fastest rate. The maximum growth is the total increase from harvest until they quit growing.

There's a lot more published information on vibrio vulnificus, and as this slide shows right here, within three-and-a-half hours they've already increased quite a bit and continue to increase for 14 hours. I bring this up just because there is more published information.

The next several slides are going to summarize some unpublished data that's just been finished up in my laboratory. This was done mostly by Jan Guch (phonetic) who is a Ph.D student with Mississippi State University, and also an employee of the National Ocean Service.

What we've done here is we've gone out into Alabama and harvested oysters each month and stored them at 26 degrees and taken samples at various times. This shows a summary of what was going on at zero hours. Usually at around 10 or less during the winter and by May through December we have levels between 100 and 1,000 per gram. Agreeing also with the data we saw from Galveston.